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Differential Accumulation of Lead by Soft Tissues of Rabbit

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Investigations related to the behavior of lead (Pb) in blood, after an acute administration of this metal showed that a large percentage of blood lead is linked to the red cells; the amount determined varies in accordance to the dose (Hursh and Mercer 1970) and the temperature (Mortenssen and Kellogg 1944). In the particular case of soft tissues, the retention of lead after an intravenous injection depends mostly on the age of the animal, and was higher in the young than in the adult (Momcilovic and Kostial 1974). On the other hand, it has been postulated that the vascularity and perfusion of each organ are the main factors that determine the different degrees of affinity for Pb of each organ (differential accumulation) under controlled conditions (Hammond 1969; Kehoe 1976).

In studies on retention of lead by soft tissues, it is reported that, in acute and chronic intoxications, the concentrations of Pb decrease in the following order: liver, kidney, heart and brain (Kehoe 1976; Barry and Mossman 1970; Barry 1975). The liver contains more lead than the other soft tissues; this might be due to the volume of blood that stays within the organ (Morgan et al 1977). Several parameters like age, temperature, perfusion, vascularity and residual blood volume could be important factors in the movement and differential accumulation of lead in blood and tissues. However, these parameters do not completely explain the quantitative differences in the retention of Pb between organs, and it is possible that other factors like the dose and the time between the administration of lead and the killing (exposure time), could have considerable importance in this process that so far has not been satisfactorily explained.

The relationships between the size of a dose of lead given intravenously and the retention of this metal by some organs, as well as the effect of the duration of exposure of an intravenously administered dose of lead on accumulation were studied in this work. The possible relationships between accumulation and the values reported for perfusion and residual blood volumes were also studied.

MATERIALS AND METHODS

The experiments included the use of twenty-one New Zealand albino rabbits (12 female and 9 male) weighing between 2.7 and 3.2 kg divided in 7 groups of three rabbits each. One group was used as a control and three groups received different doses of lead 20.95, 41.90, and 83.80 mg/kg respectively for one hour. The remaining three groups were given one dose of lead, 41.95 mg/kg, and exposed for 2, 4 and 8 hours respectively. The unanesthetized rabbits were immobilized (wrapped up in a blanket) and one marginal vein of each ear was catheterized. Heparin (0.5 U.I./kg) and lead, as a lead acetate solution pH 5.5, were administered through the catheter in the left ear. Blood samples, 2 to 2.5 ml, were taken into screw-cap test-tubes from the catheter in the right ear. The first blood sample was taken before administration of lead in order to determine the basal lead level, and four other blood samples were taken periodically throughout the exposure times. Immediately after taking the last blood sample the rabbitswere killed by decerebration and liver, heart, both kidneys and brain were removed. These organs were washed with isotonic physiological solution in order to remove the excess of blood from outside and then stored in plastic vials at -20°C.

For sample preparation, each of the organs was minced into small pieces and a 1 g sample was tranfered into a 250 ml Kjeldahl flask. For digestion of the samples 2 ml of concentrated nitric acid was added into each flask which was then placed on electric heating pads connected to rheostats and the temperature kept between 90 and 110°C until the samples were carbonized (Villarreal-Treviño et al. 1986). The digestion was repeated three times, cooling the flasks before the addition of nitric acid, until the sample became yellowish or colorless. The sample were recovered by washing the walls of the flask with deionized water to a final volume of 20 ml and the pH was adjusted to 6.5 with 1 N NaOH.

The concentrations of lead were measured using an atomic absorption spectrophotometer (Perkin Elmer Model 5000) with direct aspiration of the digested samples in a direct acetylene-air flame (Perkin Elmer 1976). Lead levels in blood were measured by chelation of lead with ammonium pyrrolidine dithiocarbamate and extraction with methylisobutyl ketone (Hessel 1968). Analyses were done in triplicate.

The precision and accuracy of the concentration of lead were estimated by their recovery from four spiked samples (99.0% $\pm$ 1.55; mean $\pm$ SE). Statistical evaluations were performed with the two-tailed Student's t-test and the results are expressed as the mean $\pm$ SE.

RESULTS AND DISCUSSION

The levels of lead in blood decreased significantly ($p<0.05$) with time after injection, with maximum levels detected 15 min after injection followed by a repid decline. Moreover, the levels of lead remaining in blood one hour after injection were higher according to the increases of the lead doses (Table 1). These results support the following conclusions: 1) blood lead concentrations decrease as a reciprocal function of time, and 2) the larger the dose of lead, the larger the amount that will be remaining in blood.

Table 2 shows that increasing exposure times at the same lead dose resulted in a decrease of lead concentrations between 1 and 8 hours in both the heart and kidney ($p<0.01$), and brain ($p<0.05$). The liver behaved differently and after 8 hours contained 45.6% more lead than that present after the first hour of exposure ($p<0.01$). Based on these result (in percentage) we suggest a classification of the tissues in three distinct groups according to the levels of lead remaining after 8 hours of exposure with respect to the levels after 1 hour of exposure: brain and heart showing a rapid decrease in the levels of lead with 10 and 11.2% of the initial levels remaining (fast disappearance); 2) kidney showing a slower decrease in lead levels with 47% of the initial levels remaining (slow disappearance); and 3) liver showing an increase of 45.6% above the initial levels of lead (maximal accumulation).

The accumulation of lead in all organs studied increased at bigger dose of lead ($p<0.05$), this confirming a dose-concentration relationship (Keller and Doherty, 1980; Aungst, et al. 1981), although the relationship was not directly proportional to the injected dose (Figure 1), moreover lead accumulation occurred in an increasing order in brain, heart, kidney, and liver.

The residual blood volume within an organ and perfusion are factors which might determine the differential accumulation of lead by soft tissues as suggested by other authors (Hammond 1969; Morgan et al. 1977). However, these factors cannot be considered as physiological parameters since they are dependent up on the handling conditions and functional state of the animals (Campistron et al. 1979). Table 3 shows that residual blood volumes are not related to lead accumulation by the organs since both brain and muscle show similar residual blood volumes (Campistron et al. 1979) but lead accumulation was 3.5 times higher in heart than in brain. Similarly, perfusion was not a determinant factor in lead retention by the organs as shown by higher perfusion values per gram for kidney (Walker et al. 1937) than for liver, but with higher lead accumulation in liver than in kidney. Perfusion values for heart (Koskinen and Bill 1983) and kidney are very similar but lead retention was 1.7 times higher for kidney than for heart.

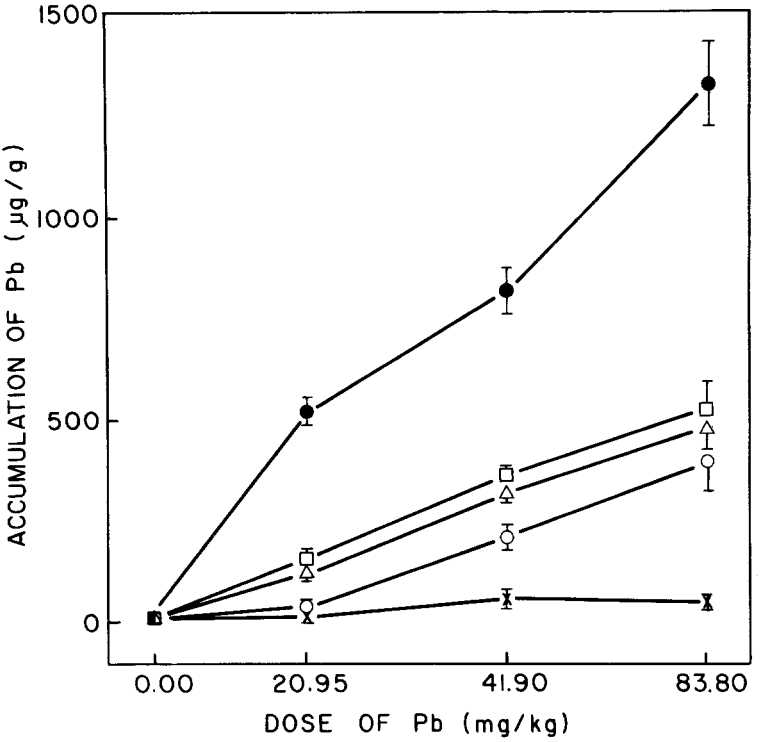

Figure 1. Accumulation of Pb by: X, Brain; O, Heart; Δ, Left kidney; □, Right kidney; ●, Liver; 1 hour after the administration of different doses of Pb. Values are expressed as the mean ± SE for 3 rabbits by dose, with three determinations for each tissue $p<0.05$.

These results suggest that 1) the decrease in the levels of lead in blood and organs is a function of time; 2) there is an increase in the levels of lead in blood and organs with an increase in the dose but the relationship is not directly proportional; 3) residual blood volume is not determinant in the differential accumulation of lead by the tissues; and 4) there is no direct relationship between regional flow and differential retention of lead by tissues. Based on the observations described above, we can advance the hypothesis that differential accumulation of lead by the organs of rabbits could be critically dependent on the inherent characteristics of each organ. These characteristics might be of a functional type as represented by the excretory function of these organs (Goyer and Rhyne 1972), the metabolic functions of the liver (Milnor 1968) or to the reabsorption of lead following the calcium pathway in the kidney (Mouw et al. 1978); and characteristics of a structural type such as, high concentrations of -SH groups of proteins and non-proteins like glutathione, which are known to bind lead (Howard 1978) and that are abundant in the liver (Jocelyn 1972). The relationships between these characteristics and lead may help to explain the differential retention of lead by the different organs, but this requires additional work.

Table 1. Blood lead level (μg/dl) in rabbits treated with lead acetate for one hour. Values are expressed as the mean ± SE for 3 rabbits by group. Comparisons between 15 and 60 min for all doses were considered significantly different at $p < 0.05$

Group: dose of Pb (mg/kg)	Minutes 0	15	30	45	60
Group 1 20.95	5.1 ± 1.7	3577.2 ± 633.7	2389.3 ± 425.7	1609.9 ± 234.7	1324.8 ± 243.5
Group 2 41.90	4.5 ± 1.0	11152.9 ± 5074.4	7356.3 ± 2604.7	4406.9 ± 468.0	2724.9 ± 0.2
Group 3 83.80	4.2 ± 0.8	12711.0 ± 3515.7	6797.0 ± 2070.9	5069.8 ± 921.2	3597.8 ± 1095.5

Table 2. Acute accumulation of lead (μg/g) by soft tissues, varying the exposure times. Dose of Pb (41.90 mg/kg as lead acetate) was injected i v, for each rabbit. Values are expressed as the mean ± S E for 3 rabbits by group, with 3 determinations for each rabbit and numbers in parentheses indicate % of the uptake at one hour (which is arbitrarily set at 100%). [a]Significantly different from one hour group ($p < 0.01$). [b]Significantly different from one hour group ($p < 0.05$)

Tissue	Exposure Times (hrs)				
	Control	1	2	4	8
Brain	4.6 ± 3.1	60.3 ± 24.6 (100)	11.5 ± 0.4 (19)	9.3 ± 1.0 (15.5)	6.0 ± 0.6[b] (10)
Heart	4.0 ± 1.2	212.3 ± 35.9 (100)	81.1 ± 8.4 (38.2)	45.7 ± 4.6 (21.5)	23.8 ± 1.8[a] (11.2)
Right kidney	11.0 ± 9.7	373.3 ± 16.8 (100)	212.4 ± 11.6 (56.8)	157.1 ± 15.7 (42)	170.6 ± 12.6[a] (45.7)
Left kidney	8.3 ± 6.5	339.6 ± 22.4 (100)	209.7 ± 9.3 (61.7)	172.4 ± 14.1 (50.7)	165.8 ± 14.5[a] (48.7)
Liver	14.0 ± 6.0	812.3 ± 33.3 (100)	794.2 ± 42.3 (97.7)	1102.2 ± 30.4 (135.6)	1183.0 ± 68.1[a] (145.6)

Table 3. Perfusion, residual volume of blood and accumulation of Pb values in soft tissues of rabbits. [a]Walker et al (1937), [b]White et al. (1967) and [c]Koskinen and Bill (1983), 1 ml 1 g; [d]Campistron et al (1979), [e]Accumulation values are expressed as the mean ± S E for 3 rabbits, by triplicate. The dose of Pb (41.90 mg/kg) as lead acetate) was injected i v, for each animal.

Tissues	Perfusion (ml/min/g)	[d]Residual Volume (ml/g)	[e]Accumulation (μg/g)
Heart	[c]4.71 ± 0.36	0.030 ± 0.0057	212.3 ± 35.9
Kidneys	[a]5.00	0.286 ± 0.034	373.3 ± 16.8
Liver	[b]0.37	0.176 ± 0.019	812.3 ± 33.3
Brain	[c]1.01 ± 0.06	0.025 ± 0.0041	60.3 ± 24.6

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